# A systematic review of Hepatitis B virus (HBV) drug and vaccine escape mutations in Africa: a call for urgent action

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**Short title:** Drug and vaccine resistance in HBV in Africa

**Keywords:** Hepatitis B virus, treatment, antiviral, nucleotide analogues, mutations, drug resistance, vaccine escape, Africa

**ABSTRACT:**

International sustainable development goals for the elimination of viral hepatitis as a public health problem by 2030 highlight the pressing need to optimize strategies for prevention, diagnosis and treatment. Selected or transmitted resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) in hepatitis B virus (HBV) may reduce the success of existing treatment and prevention strategies. These issues are particularly pressing for many settings in Africa where there is high HBV prevalence and co-endemic HIV infection, but lack of robust epidemiological data and limited education, diagnostics and clinical care. The prevalence, distribution and impact of RAMs and VEMs in these populations are neglected in the current literature. We therefore set out to assimilate data for sub-Saharan Africa through a systematic literature review and analysis of published sequence data, and present these in an on-line database (https://livedataoxford.shinyapps.io/1510659619-3Xkoe2NKkKJ7Drg/). The majority of the data were from HIV/HBV coinfected cohorts. The commonest RAM was rtM204I/V, either alone or in combination with compensatory mutations, and reported in both treatment-naïve and treatment-experienced adults. We also identified the suite of mutations rtM204V/I + rtL180M + rtV173L that is reported in association with vaccine escape, in over 1/3 of cohorts. Although tenofovir has a high genetic barrier to drug resistance, it is of concern that emerging data suggest polymorphisms that may be associated with resistance, although the precise impact of these is unknown. Overall, there is an urgent need for improved diagnostic screening, enhanced laboratory assessment of HBV before and during therapy, and sustained roll out of tenofovir in preference to lamividine. Further data are urgently needed in order to inform population and individual approaches to HBV diagnosis, monitoring and therapy in these highly vulnerable settings.

**Author's summary**

The Global Hepatitis Health Sector Strategy is aiming for the elimination of viral hepatitis as a public health threat by 2030. However, drug and vaccine resistance may reduce the success of existing treatment and prevention strategies. In the current literature, the prevalence, distribution and impact of hepatitis B drug and vaccine resistance in many settings in Africa are neglected, despite the high prevalence of hepatitis B and co-endemic HIV infection. This systematic review describes the frequency, prevalence and co-occurrence of mutations associated with hepatitis B drug and vaccine resistance in Africa. The findings suggests a high prevalence of these mutations in some populations in sub-Saharan Africa. Scarce resources have contributed to the lack of diagnostic screening, inconsistent supply of hepatitis B drugs, and poor access to clinical monitoring, all of which contribute to drug and vaccine resistance. Sustainable long-term investment is required to expand consistent drug and vaccine supply, to provide screening infection and for drug resistance, and to provide appropriate targeted clinical monitoring for treated patients.

## ABBREVIATIONS

- 3TC - lamivudine
- ADV – Adefovir
- ART – antiretroviral therapy
- AZT – zidovudine
- CHB – chronic hepatitis B infection
- DNA – deoxyribonucelic acid
- ETV – entecavir (ETV)
- HBsAg – Hepatitis B surface antigen
- HBV – Hepatitis b virus
- LdT – telbivudine
- NA – Nucleos(t)ide analogue
- ORF – overlapping reading frame
- Pol – polymerase
- RAM – Resistance Associated Mutation
- RNA – ribonucleic acid
- RT – reverse transcriptase
- TAF – tenofovir alafenamide
- TDF – tenofovir disoproxil fumarate
- VEM – Vaccine escape mutation
- WHO – World Health Organisation

## INTRODUCTION

In 2015, the World Health Organisation (WHO) estimated that 3.5% of the world's population (257 million people) were living with Hepatitis B virus (HBV) infection, resulting in 887,000 deaths each year, mostly from complications including cirrhosis and hepatocellular carcinoma (HCC) (1). United Nations Sustainable Development Goals set out the challenge of 'elimination of viral hepatitis as a public health threat by the year 2030' (2). One of the existing strategies in the elimination toolbox is use of antiviral drugs in the form of nucleos(t)ide analogues (NAs). Suppression of viraemia not only reduces inflammatory liver disease in the individual receiving treatment but also reduces the risk of transmission. However, the emergence of HBV resistance-associated mutations (RAMs) is a potentially significant concern for the success of this strategy.

Africa is the continent with the second largest number of individuals with chronic HBV infection (CHB), having 6.1% of the adult population infected (1). However, there is very little commitment and resources invested into the burden of this disease, and many barriers are contributing to the epidemic (3,4). Globally, less than 10% of the population with CHB infection are diagnosed, with an even smaller proportion on treatment (1,4). This proportion is likely to be even lower in Africa. The situation in Africa is further complicated by the substantial public health challenge of coendemic human immunodeficiency virus (HIV) and HBV; high coinfection rates worsen the prognosis in dually infected individuals (5). There is also a lack of robust epidemiological data on HBV from Africa (3,4).

Widespread use of antiretroviral therapy (ART) for HIV, incorporating NAs that also have activity against HBV, may have an impact on HBV both through improved rates of viraemic suppression, and also potentially by driving the selection of RAMs. The WHO recommends HBsAg screening for all HIV-1 infected individuals prior to ART initiation and for all pregnant women during antenatal visits, to improve the clinical outcomes of people living with CHB and to reduce the incidence of new cases (6). However, screening of HBsAg is not routinely performed in many settings in Africa due to cost and lack of availability. HBV infected patients either remain untreated (in the setting of monoinfection), or are exposed to antiviral drugs without proper monitoring, putting them at risk of developing RAMs (in the setting of HIV coinfection) (4,7–10).

HBV is a DNA virus that replicates via an RNA intermediate, with reverse transcriptase (RT) catalysing the transcription of RNA into DNA (7). NAs that inhibit RT are therefore used to prevent HBV replication, including lamivudine (3TC), entecavir (ETV) and tenofovir disoproxil fumarate (TDF), with historical use of other agents including telbivudine (LdT) and adefovir (ADV)(6,11). Choice of agent is determined by a number of factors including potency, availability, cost, side-effects, drug interactions, safety in pregnancy, and genetic barrier to resistance (4). Emergence of mutations happens because the RT enzyme lacks proofreading function required to repair errors during transcription (7); when these mutations confer a selective advantage by allowing the virus to escape the effect of drug therapy, they will become amplified in the viral population. Some RAMs confer resistance to one agent only, while others are associated with resistance to several agents (Fig 1). In Africa, the choice of agent is usually limited to 3TC and/or TDF.

**Fig 1: HBV drug resistance associated mutations (RAMs), vaccine escape mutations (VEMs) and mutations associated with Hepatitis B immunoglobulin (HBIg) resistance.** HBV genes are shown in the coloured ovals. TDF = tenofovir, ETV = entecavir, 3TC = lamivudine. This figure incorporates data from eight studies; three were identified by the systematic review presented in this manuscript (45,49,59) and five from the wider literature (7,13,14,20,27).

3TC was originally seen as a major breakthrough in treating HBV (12). However, it is now known to have a low genetic barrier to resistance and its long-term effectiveness is limited as a result of resistance mutations in the 'YMDD' motif (tyrosine, methionine, aspartate, aspartate; amino acids 203-206) in domain C of the viral polymerase (Pol) and associated upstream compensatory mutations in Pol domains A, B and in the B-C interdomain (7,13,14). Among chronic HBV monoinfected patients, incidence of HBV resistance to 3TC is as high as 20% per year, whereas in HIV/HBV coinfected patients, this can reach 90% over 5 years of treatment(5). 3TC has also been associated with the induction of cross-resistance to emtricitabine (FTC), LdT, and ETV, thus reducing the options for subsequent treatment (10).

TDF is widely used in treatment of both HIV and HBV and is generally well tolerated. TDF has a high genetic barrier to resistance and maintains effective suppression of HBV in both monoinfected and HIV/HBV coinfected individuals (5,7,10,15,16). Although it has a recognised association with nephrotoxicity in HIV treatment, current literature suggests it may be better tolerated in HBV infection (11). African populations have a higher background of renal disease (17) and could be potentially

more vulnerable to nephrotoxitiy from TDF(18). A tenofovir prodrug, tenofovir alafenamide (TAF), delivers equally potent viraemic suppression at lower plasma levels, and is therefore thought to be associated with reduced nephrotoxicity(19). However, TAF is not widely available. TDF resistance is not well characterised, but there are emerging data from *in vitro* studies associating Pol mutations rtA194T and rtN236T with decreased susceptibility (11,15). Virological breakthrough on TDF therapy has been reported in two patients harbouring rtS78T/sC69 mutations (20), and in another patient with multisite polymerase mutations; rtL80M, rtL180M, rtM204V/I, rtA200V, rtF221Y, rtS223A, rtT184A/L, rtR153Q, and rtV191I (21). The significance of these mutations needs to be further explored in clinical studies.

Current guidelines recommend the use of first-line ART regimens containing TDF in patients coinfected with HIV and HBV (22). In accordance with this, there is evidence of a recent increase in the prescription of TDF in sub-Saharan Africa (sSA) (23). However, it is likely that many ART regimens in sSA continue to include 3TC as the only agent active against HBV (7,8). In South Africa, second line ART substitutes TDF for Zidovudine (AZT) leaving only 3TC coverage for HBV. Among HBV/HIV coinfected children in South Africa treated with regimens including 3TC and/or TDF, HBV viraemia has been demonstrated highlighting potential underlying HBV drug resistance (24).

ETV is another active agent, and is safe and well tolerated. However it is not active against HIV and therefore has to be added to ART regimens rather than being part of the primary backbone, is not recommended in pregnancy, and is not routinely available in most African settings(25). Resistance arises more commonly in the

context of prior 3TC exposure (11,26), which may limit its future potential in Africa, particularly in HIV endemic populations.

As a component of the Expanded Programme on Immunization (EPI), HBV preventive vaccines have been rolled out in Africa since 1995 (4). Mutations in HBV Pol can also lead to amino acid changes in the Surface (S) protein due to overlapping reading frames (ORFs) in the genome (14). Changes in the S protein can result in vaccine escape mutants (VEMs) which can cause infection among vaccinated individuals(14,27), and also diagnostic escape mutations which result in false negative HBsAg testing (14). S protein mutation sG145R has been identified as the major VEM and recently other mutations in S protein have been associated with immune escape (14) (Fig 1). There are no data for VEMs in Africa, but in other settings of high endemicity VEMs can be common, as evidenced by a reported prevalence of 28% in vaccinated HBV-infected children in Taiwan (28).

To date, no systematic review has assessed the geography and prevalence of HBV RAMs and VEMs in Africa. An understanding of the extent to which these mutations circulate in Africa is essential to improving HBV therapy in patients with and without HIV coinfection. We therefore set out to describe the frequency, co-occurrence and distribution of RAMs and VEMs in Africa, and to suggest whether changes are needed in recommendations for laboratory diagnostics and/or approaches to drug therapy or vaccine deployment. This will underpin further research to identify and track relevant mutations in these populations.

## METHODS

### Search strategy

Between October 2017 and January 2018, we searched the published literature, in MEDLINE (PubMed; https://www.ncbi.nlm.nih.gov/pubmed), SCOPUS (https://www.elsevier.com/solutions/scopus) and EMBASE (https://www.elsevier.com/en-gb/solutions/embase-biomedical-research). Our search strategy is detailed in S1 Table and S1 Fig. The earliest paper we identified on HBV drug resistance in Africa was published in 2007. We reviewed the titles and abstracts matching the search terms and only included those relating to drug or vaccine resistance in HBV infection, including only those that presented original data and had undergone peer review. All retrieved articles were in English, therefore no exclusion in relation to language was required.

For each publication we recorded reference, publication year, study design, sample size, study population, proportion of participants who tested HBsAg+ or HBV DNA+, country, year(s) of specimen collection, genotype identified, antiviral treatment, sequencing method, gene sequenced, number of sequenced samples, participant recruitment site and sequence accession number. Data were curated using MS Excel software (Microsoft, Redmond, WA).

### RAMs reported in published sequences not represented in primary studies

We expanded our search for evidence of RAMs by identifying publicly available HBV sequences from Africa, that had not been included in the results of our primary literature search. We used both the Hepatitis B Virus database

(https://hbvdb.ibcp.fr/HBVdb/)(29) and Hepatitis Virus Diversity Research Alignments database (http://hvdr.bioinf.wits.ac.za/alignments/)(30).

**Analysis**

In order to determine the prevalence of RAMs and VEMs, we first reported these using the denominator (total number of HBV positive patients) and numerator (total number of HBV positive patients with the specified mutation) as reported in published studies. We also pooled data by country in order to provide regional estimates. Downloaded sequences were managed using Sequence editor, database and analysis platform, SSE version 1.3, for analysis (31).

**Data visualisation**

We developed an R package, gene.alignment.tables, for the visualisation of the sequence data in this study; this is available on Github (32) and can be used for visualising generic gene sequence datasets. The package was developed by University of Oxford's Interactive Data Network and a specific instance of the visualisation is hosted as a Shiny app which can be viewed here: https://livedataoxford.shinyapps.io/1510659619-3Xkoe2NKkKJ7Drg/ (33).

**RESULTS**

The initial search yielded 56 articles in MEDLINE, 150 in SCOPUS and 150 in EMBASE. Of these; 32, 136 and 119 were excluded from search results of MEDLINE, SCOPUS and EMBASE respectively, as they did not did not meet the inclusion criteria. After de-duplication, 37 articles were included: 27 articles identified from MEDLINE, SCOPUS and EMBASE were identical; five unique articles were

included from EMBASE, four from SCOPUS and one from MEDLINE. A total of 37 articles were downloaded in full (S1 Fig; S2 Table).

**Study characteristics**

Epidemiological data for HBV represented by the 37 studies we identified are summarised in Table 1. Studies included were from Southern Africa (Botswana, Mozambique, South Africa, Zambia and Zimbabwe), East Africa (Ethiopia, Kenya, Malawi, Sudan and Uganda), West Africa (Cote d'Ivoire, Gambia, Ghana, Guinea-Bissau and Nigeria) and Central Africa (Cameroon, Gabon). There was considerable heterogeneity in recruitment protocols and exposure to anti-viral treatment. Twenty-six studies recruited from hospitals, three studies recruited from the community (8,34,35) and eight studies did not specify where recruitment was undertaken (10,36–42). All studies were observational.

**Table 1: Prevalence of HBsAg, HBeAg and HDV coinfection**

| | Study (Reference) | Country | Characteristics of study population | HIV co-infection status of cohort[a] | HBV prevalence in this cohort (reported as HBsAg prevalence unless otherwise specified) | HBeAg prevalence among HBV-positive individuals |
|---|---|---|---|---|---|---|
| **East Africa** | Deressa et al 2017 (42) | Ethiopia | Patients attending outpatient ART clinic at tertiary referral university hospital | + | 17/308 (6%) | Not reported |
| | Hundie et al 2016 (33) | Ethiopia | Stored plasma samples from HBV infected blood donors obtained from blood bank centres | ± | 391/391 (100%) | Not reported |
| | Day et al 2013 (7) | Kenya | Longitudinal cohort study of female sex workers in an urban setting | + | 11/159 (7%) | 6/11 (55%) |
| | Kim et al 2011 (8) | Kenya | Individuals from an urban centre enrolled in randomised controlled trial of adherence to ART | + | 27/389 (7%) | 24/27 (89%) |
| | Mabeya et al 2017 (44) | Kenya | Individuals seeking treatment at the comprehensive HIV Clinic at tertiary referral university hospital | + | 29/400 (7%) | Not reported |
| | Auodjane et al 2014 (45) | Malawi | Individuals starting ART treatment at a tertiary referral university hospital | + | 133/1117 (12%) | 67/133 (50%) |
| | Galluzzo et al 2012 (37) | Malawi | Pregnant women enrolled in a PMTCT study on safety and pharmacokinetics of antiretroviral drugs | + | 21/21 (100%) | 7/21 (33%) |
| | Mahgoub et al 2011 (34) | Sudan | Plasma samples from blood donors from capital city in Sudan | ± | 16/404 (4%) | Not reported |
| | Yousif et al 2014 (39) | Sudan | Individuals seeking treatment at a AIDS care unit and HIV treatment centre | + | 96/358 (27%)[b] | 32/ 50 (64%) |
| | Calisti et al 2015 (46) | Uganda | All HIV patients attending a regional referral hospital | + | 109/2820 (4%) | Not reported |

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| **West Africa** | Boyd et al 2015 (47) | Cote d'Ivoire | Individuals enrolled in randomised multi centre trials of benefits and risks of early ART initiation | + | 259/ 2465 (11%) | 39/168 (23%) |
| | Archampong et al 2017 (48) | Ghana | Serum samples from HBV-HIV co-infected patients collected at tertiary referral university hospital | + | 235/235 (100%) | Not reported |
| | Chadwick et al 2012 (49) | Ghana | Stored sera from all adult patients attending the HIV clinic at a tertiary referral university hospital | + | 143/371 (39%) | Not reported |
| | Geretti et al 2010 (50) | Ghana | Paired serum and plasma samples collected from HIV-infected patients attending a tertiary referral university hospital | + | 140/838 (17%) | 37/140 (26%) |
| | Ndow et al 2017 (51) | Gambia | Individuals attending HIV clinic | + | 23/187 (12%) | Not reported |
| | Stewart et al 2011 (36) | Gambia | Individuals receiving HAART; recruitment site not specified | + | 70/ 570 (12%) | 6/21 (29%) |
| | Langhoff Honge et al 2014 (52) | Guinea Bissau | Patients attending outpatient ART clinic at tertiary referral university hospital | + | 94/576 (16%) | 16/94 (17%), HDV prevalence : 18/72 (25%) |
| | Faleye et al 2015 (64) | Nigeria | Pregnant women attending antenatal clinics from two tertiary university hospitals | ± | 15/272 (6%) | Not reported |
| **Central Africa** | Gachara et al 2017 (53) | Cameroon | Patients attending outpatient ART health centre | + | 20/337 (6%)[c] | Not reported |
| | Kouanfack et al 2012 (54) | Cameroon | Patients attending outpatient ART clinic at tertiary hospitals | + | 54/552 (10%) | Not reported |
| | Magoro et al 2016 (55) | Cameroon | Patients attending outpatient ART health centre | + | 116/445 (26%) | 16/102 (16%) |
| | Bivigou-Mboumba et al 2016 (56) | Gabon | Patients attending outpatient ART clinic | + | 71/762 (9%) | Not reported |
| | Bivigou-Mboumba et al 2018 (43) | Gabon | Patients attending HIV care centers | + | 43/487 (9%) | Not reported |

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|---|---|---|---|---|---|---|
| **Southern Africa** | Anderson et al 2015 (40) | Botswana | Stored plasma samples of HIV/HBV co-infected individuals collected from studies conducted in a Research Institution | + | 81/81 (100%) | Not reported |
| | Matthews et al 2015 (62) | Botswana | Women attending antenatal and paediatric clinics | ± | 17/443 (4%) | 16/60 (27%); HDV: negative |
| | Chambal et al 2017 (57) | Mozambique | Patients attending outpatient ART health centre | + | 47/518 (9%) | Not reported |
| | Wandeler et al 2016 (58) | Mozambique | Individuals starting ART treatment at urban clinic in Mozambique and rural clinic in Zambia | + | 78/1032 (8%) | 24/168 (14%) |
| | Andersson et al 2013 (63) | South Africa | Stored serum of women infected with HIV enrolled in an Antenatal Sentinel HIV and Syphilis Prevalence Survey | ± | 97/3089 (3%) | 17/94 (18%); HDV: negative |
| | Amponsah-Dacosta et al 2015 (35) | South Africa | Stored serum of individuals exposed to HBV participating in a health facility-based hepatitis B serosurvey conducted at a provincial level. | ± | 33/201 (16%) | Not reported |
| | Amponsah-Dacosta et al 2016 (41) | South Africa | Individuals due to HAART initiation enrolled in longitudinal study | + | 5/5 (100%) | 5/5 (100%) |
| | Hamers et al 2013 (9) | South Africa | Individuals enrolled in a multicentre prospective study of ART resistance monitoring | + | 37/175 (21%) | Not reported |
| | Gededzha et al 2016 (65) | South Africa | Stored sera from HBV infected individuals attending a tertiary referral university hospital | ± | 8/9 (89%) | Not reported |
| | Makondo et al 2012 (38) | South Africa | Stored sera from HIV infected individuals prior to ART initiation, recruitment site not specified | + | 71/298 (24%)[b] | Not reported |

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| | Matthews et al 2015 (62) | South Africa | Women attending antenatal and paediatric clinics in South Africa and Botswana | ± | 49/507 (10%) | Not reported; HDV: negative |
| | Powell et al 2015 (61) | South Africa | Stored serum samples of individuals infected with HIV receiving care at a tertiary university hospital | + | 37/394 (9%) | Not reported |
| | Selabe et al 2007 (66) | South Africa | Individuals infected with HBV admitted at tertiary University hospital | ± | 35/35 (100%) | Not reported |
| | Selabe et al 2009 (67) | South Africa | Individuals infected with HBV admitted at tertiary University hospital | - | 17/17 (100) | 9/17 (53%) |
| | Wandeler et al 2016 (58) | Zambia | Individuals starting ART treatment at urban clinic in Mozambique and rural clinic in Zambia | + | 90/797 (11%) | 24/168 (14%) |
| | Hamers et al 2013 (9) | Zambia | Individuals enrolled in a multicentre prospective study of ART resistance monitoring | + | 55/523 (11%) | Not reported |
| | Baudi et al 2017 (59) | Zimbabwe | Stored plasma samples of individuals attending HIV support clinic | + | 19/176 (11%) | Not reported |

HBsAg, HBeAg and HDV coinfection prevalence across 37 studies identified by a systematic literature search of HBV resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) from African cohorts published between 2007 and 2017 (inclusive).

[a] HIV status is designated '+' whole cohort HIV-positive; '±' some of cohort HIV-positive; '-'none of cohort HIV-positive

[b] HBV prevalence in these cohorts was reported using HBV DNA detection rather than HBsAg

[c] Occult HBV prevalence reported in these cohorts

Study populations were categorised as follows:

- HBV/HIV coinfected patients: (n=28 studies), (8–10,37–40,42–62);
- HBV infected with and without HIV coinfection: (n=8 studies), (34–36,63–67);
- Chronic HBV monoinfection: (n=1 study), (68).

Antiviral treatment exposure varied as follows:

- Treatment-naïve: (n=8 studies), (39–41,58–60,63,67);
- 3TC-based regimen only: (n=11 studies), (8,9,37,38,42,43,46,50,55,57,67);
- Regimens including 3TC or TDF: (n=8 studies), (9,42, 43,44,46,47,51,63);
- Mixed regimen where some received 3TC, others TDF, while others left untreated; (n=4 studies),(44,49,56,66);
- Treatment regimen not specified: (n=6 studies), (33–35,53,61,64).

HBV amino acid polymorphisms were studied from within the following proteins;

- Pol only (n=13 studies), (8,9,37,45,46,49,50,52,53,55,59,63,67); only one of these used a deep sequencing method (46);
- S only (n=3 studies), (36,64,65);
- Pol and S (n=12 studies),(10,34,38,41,44,47,48,51,54,56,58,62);
- Polymerase, S and PC/BCP (n=4 studies), (40,43,57,68);
- S and PC/BCP region (n=3 studies), (35,39,60);
- Whole genome (n=2 studies), (42,66).

All studies, except for two (50,67), specified the HBV genotype (S2 Table & S3A Fig).

**Prevalence of HBsAg, HBeAg and HDV co-infection**

The prevalence rates of HBsAg in these study cohorts ranged from 3%-26%; however, the populations included were highly selective and therefore not necessarily representative of the general population, particularly as a result of a strong bias towards HIV-infection (Table 1). Only three studies included in this

review reported on HDV prevalence: two studies did not detect any HDV antibodies (63,64), whereas the other study reported a HDV prevalence of 25% in Guinea-Bissau (53).

**RAMs identified in African cohorts**

The co-occurrence and distribution of HBV RAMs and VEMs are summarised according to the region where they were identified (Fig 2). This illustrates the patchy and limited data that are available, with South Africa, Ghana and Cameroon best represented, but with large areas (especially in northern and central Africa) not represented at all in the literature.

**Figure 2: Annotated map to summarise HBV drug Resistance Associated Mutations (RAMs) and Vaccine Escape Mutations (VEMs).** Mutations identified from 33 studies of African cohorts published between 2007 and 2017 (inclusive). Four studies identified by our systematic literature review were not represented here as they did not report any RAMs.

Although 35 studies specified the HBV genotype, it was only possible to group RAMs according to genotype in fourteen studies (8,9,37,39,40,43–45,47,53,56,57,59,68) (S3B Fig).The remaining 21 studies generally reported the genotypes identified, however they were not specific on which genotype inidividuals with RAMs were infected with.

We have developed an interactive tool to display the genomic positions of RAMs identified through our literature review alongside relevant metadata. This can be

accessed on-line here: https://livedataoxford.shinyapps.io/1510659619-3Xkoe2NKkKJ7Drg/ (33).

Overall, the most prevalent RAM was rtM204V/I in both treatment experienced and treatment naïve patients and occurring either alone or in combination with other polymorphisms rtL80I/V, rtV173L, rtL180M, rtA181S, rtT184S, rtA200V and/or rtS202S (Fig 3, S3 Table & S4 Table). This mutation was present in 29 studies at a highly variable prevalence of between 0.4% (49) and 76% (68). Across all cohorts, the mutation was present in 208/2569 (8%) of all individuals represented. The mutation, by itself, was most prevalent in South Africa; on pooling data for three studies from this setting, it was present in both treatment experienced and treatment naïve patients (n=13/17, 76% (68) and n=16/72, 22% (62,67) respectively). In addition to South Africa, rtM204I/V was also frequent in Malawi among treatment experienced patients (n=24/154, 16% (38,46)) (Fig 3), and in genotype non-A infection: in this setting, the mutation was detected in genotype C infection (n=2/17, 12% (68)) (S3B Fig).

**Figure 3: Prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins among HBV infected patients in Africa.**

These data are derived from 27 studies of HBV drug resistance in Africa published between 2007 and 2017 (inclusive). The countries represented are listed in alphabetical order. A detailed summary of RAMs identified from each study is presented in fig 2, and in Suppl Tables 3 and 4. Prevalence of RAMs for a specific country was determined by grouping all studies from that country that reported a specific mutation; the denominator was the total number of individuals infected with

HBV from these studies and the numerator was the total number of individuals with that specific mutation from these studies.

Panel A: treatment naïve;

Panel B: treatment experienced.

rtM204I/V mutation by itself confers resistance to 3TC, and may also be associated with reduced efficacy to TDF when in combination with A194T, and with vaccine escape in combination with L180M and V173L. Although TDF has a high genetic barrier to resistance, and is associated with reliable suppression of HBV viraemia (7,10,15,16), mutations rtN236T and rtA194T, which have been linked with resistance to both TDF and ADV (7), have been identified in Southern Africa in both treatment naïve (59) and treatment experienced (10) patients.

WHO guidelines recommend a first-line regimen including TDF in HIV/HBV coinfected patients(6), and the South African Department of Health HIV/AIDS treatment guideline included TDF as first-line regimen from 2010 (69), however we found a minority of studies (9/37, 24%) reporting TDF-containing regimens for HIV/HBV coinfected individuals. As anticipated, most of the studies that did use TDF were carried out after 2010, whereas those that used 3TC were generally earlier (S2 Table).

From this dataset, it is difficult to ascertain whether RAMs are genuinely more prevalent in genotype A infection, or this simply reflects enrichment of genotype A in sub-Saharan African populations (S3 Fig). Interpreting RAMs according to sub-genotypes was difficult since most studies did not specify sub-genotype and others

did not indicate which RAMs were identified in which genotype. Of concern is the detection of RAMs even in treatment naïve individuals (Fig 3 & S3 Table), suggesting that RAMs are being transmitted. A study in South Africa that recruited 3TC-naïve HBV infected adults with or without HIV, reported rtM204I in 13/35 (37%) individuals (67).

**HBV RAMs in published sequences from Africa**

We searched the Hepatitis B Virus database and GenBank to identify HBV sequences derived from Africa, from studies not already included in our review. We identified additional 69 isolates: 23 had undergone full length genome sequencing whereas 46 isolates represented either the polymerase (n=3) or S region (n= 43) of the HBV genome (Table 2). We excluded fourteen studies already identified by our literature review that had submitted their sequences to GenBank (S2 Table) to avoid duplication of results. RAMs in the additional 69 isolates were as follows:

- rtM204V in genotype A (2/69, 2.9% of sequences), this occurred in combination with rtL180M;
- rtM204V + rtL180M in genotype E (1/69, 1.5%);
- rt180M + rtA181V in genotype E (1/69 (1.5%);
- rtQ215S identified in genotype D (4/69, 5.8%).

All these mutations are associated with 3TC resistance, rtA181V has also been associated with reduced susceptibility to TDF (7,13).

In the S gene, the most prevalent mutations were:

- sD144A/E/G occurring in genotype A (6/69, 8.7%), D (10/69, 14.5%) and E (7/69, 10.1%) associated with VEM;
- sI110L occurring in genotype A (3/69, 4.3%), D (4/69, 5.8%) and E (11/69,

15.9%) associated with immunoglobulin resistance.

**Table 2: HBV drug resistant mutations (RAMs) identified from HBV genome sequences from Africa downloaded from the Hepatitis B Virus database (https://hbvdb.ibcp.fr/HBVdb/)** (31) **and GenBank database (http://hvdr.bioinf.wits.ac.za/alignments/)** (32)

| HBV Protein | HBV Genotype | Position and nature of the mutation | Number (%) of HBV sequences with mutation | Accession number | Region sequenced | Country of origin |
|---|---|---|---|---|---|---|
| Polymerase (Pol) | A | rtL180M | 2/69 (2.9%) | KM519454 | Full length | South Africa |
| | | | | FM199980 | Full length | Rwanda |
| | | rtM204V | 2/69 (2.9%) | KM519454 | Full length | South Africa |
| | | | | FM199980 | Full length | Rwanda |
| | | rtI233V | 1/69 (1.4%) | HM535205 | Full length | Zimbabwe |
| | D | rtV214A | 1/69 (1.4%) | FJ904395 | Polymerase | Tunisia |
| | | rtQ215S | 4/69 (5.8%) | FJ904414 | Full length | Tunisia |
| | | | | FJ904431 | Full length | Tunisia |
| | | | | FJ904436 | Full length | Tunisia |
| | | | | FJ904438 | Full length | Tunisia |
| | E | rtV173L | 1/69 (1.4%) | KF849723 | Full length | Angola |
| | | rtL180M | 2/69 (2.9%) | KF849720 | Full length | Angola |
| | | | | KF849723 | Full length | Angola |
| | | rtA194T | 1/69 (1.4%) | GQ161771 | Full length | Guinea |
| | | rtM204V | 1/69 (1.4%) | KF849723 | Full length | Angola |
| | | rtA181V | 1/69 (1.4%) | KF849720 | Full length | Angola |
| | | rtN238D | 2/69 (2.9%) | HM363566 | Polymerase | Nigeria |
| | | | | HM363587 | Polymerase | Nigeria |
| Surface (S) | A | sI110L | 3/69 (4.3%) | KY493896 | S | Cameroon |
| | | | | KP168431 | Full length | Kenya |
| | | | | AY233286 | Full length | South Africa |
| | | sP120S | 1/69 (1.4%) | KX648547 | S | Zimbabwe |
| | | sG129R | 1/69 (1.4%) | FN547352 | S | Cameroon |
| | | sT126A | 1/69 (1.4%) | JN182330 | S | South Africa |
| | | sD144A/E/G | 6/69 (8.7%) | FN547249 | S | Cameroon |
| | | | | KX493873 | S | Cameroon |
| | | | | FM199980 | Full length | Rwanda |
| | | | | FM200180 | S | Rwanda |
| | | | | FM200189 | S | Rwanda |
| | | | | KF467020 | S | South Africa |
| | | sG145R | 1/69 (1.4%) | FM200185 | S | Rwanda |
| | | sC149R | 1/69 (1.4%) | KF476024 | S | South Africa |

| | | | | | | |
|---|---|---|---|---|---|---|
| | D | sI110L | 4/69 (5.8%) | AB561830 | S | Egypt |
| | | | | KX357627 | Full length | Ethiopia |
| | | | | FJ904429 | S | Tunisia |
| | | | | KJ416196 | S | Tunisia |
| | | sP120S | 1/69 (1.4%) | KX357636 | Full length | Ethiopia |
| | | sM133T | 1/69 (1.4%) | KM108592 | S | Sudan |
| | | sD144E | 10/69 (14.5%) | FJ904427 | Full length | Tunisia |
| | | | | FN547165 | S | Cameroon |
| | | | | FN547179 | S | Cameroon |
| | | | | FN547239 | S | Cameroon |
| | | | | FN547255 | S | Cameroon |
| | | | | FN547258 | S | Cameroon |
| | | | | FN547262 | S | Cameroon |
| | | | | FN547281 | S | Cameroon |
| | | | | FN547318 | S | Cameroon |
| | | | | FN547319 | S | Cameroon |
| | E | sI110L | 11/69 (15.9%) | KY494047 | S | Cameroon |
| | | | | AM494711 | S | Central Africa Republic |
| | | | | AM494720 | S | Central Africa Republic |
| | | | | AM494725 | S | Central Africa Republic |
| | | | | AM494727 | S | Central Africa Republic |
| | | | | JQ972822 | S | Central Africa Republic |
| | | | | AB205190 | Full length | Ghana |
| | | | | GQ161756 | S | Guinea |
| | | | | GQ161768 | Full length | Guinea |
| | | | | GQ161795 | | Guinea |
| | | | | DQ060822 | Full length | South Western Africa and Madagascar |
| | | sT126N | 1/69 (1.4%) | HM363608 | S | Nigeria |
| | | sP120S | 1/69 (1.4%) | HM363599 | Full length | Nigeria |
| | | sM133T | 3/69 (4.3%) | HM363603 | S | Nigeria |
| | | | | KF170751 | S | Sudan |
| | | | | KF170752 | S | Sudan |
| | | sD144E | 7/69 (10.1%) | FN547300 | S | Cameroon |
| | | | | KY494047 | S | Cameroon |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | AM494719 | S | Central African Republic |
| | | | | AM494726 | S | Central African Republic |
| | | | | FN594756 | Full length | Niger |
| | | | | HM363565 | Full length | Nigeria |
| | | | | HM363590 | S | Nigeria |
| | | G145A/R | 3/69 (4.3%) | KY493921 | S | Cameroon |
| | | | | AB205327 | S | Ghana |
| | | | | AM494741 | S | Central African Republic |

**VEMs**

VEMs were identified in Central, East, West and Southern Africa (Fig. 2). However, it was not possible to ascertain whether individuals harbouring these mutations had been vaccinated against HBV infection. The most common VEM was the triple mutation rtV173L + rtL180M + rtM204I/V, found in the Pol gene. This suite of mutations was identified in 14 studies (37,43–52,54–56), at a pooled prevalence of 4% (57/1462). Another significant VEM, sG145K/R (14), was identified in six studies (35,40,44,49,56,65) and sM133L/T, associated with VEM, immunoglobulin and diagnostic escape mutation(41,49), was identified in seven studies (34,40,41,44,49,60,65) (Fig. 2).

## DISCUSSION

### Summary

To our knowledge, this is the first systematic review that assesses RAMs and VEMs for HBV in Africa. The high rates of HBV infection among HIV infected individuals in some locations including Cameroon (56) and South Africa (10) could be an indication that HBV infection has been previously under-reported, possibly due to lack of

routine screening, poor awareness, stigma, high costs and limited clinical and laboratory infrastructure (4,8–10,38,50). The literature suggests a widespread exposure of the HIV-infected population to 3TC-based treatment. Although this may be changing over time, the introduction of TDF-based regimens for HIV treatment has been slow and inconsistent, and TDF monotherapy is not consistently available for HBV infection in the absence of HIV.

In keeping with other settings, the most common RAM identified here was rtM204I/V, either alone or in combination with compensatory mutations rtL180M ± rtV173L. Of concern, rtM204V/I was seen in 76%, (68) of treatment experienced patients and 22% of treatment naïve patients (62,67) in South Africa. A review of worldwide incidence of RAMs among treatment naïve patients also described rtM204V/I as the most frequent, but with a much lower prevalence of 5% (70). Likewise, a European study demonstrated that the most frequent primary mutation was rtM204V/I, found in 49% of treatment experienced patients (71), while in China rtM204I, rtN236T and rtL180M+rtM204V+rtV173L/rtS202G were also the most prevalent RAMs (72).

The triple mutation rtM204V + rtL180M + rtV173L has been identified in East, West and Central Africa (37,46–48,50,51,55). This combination of polymorphisms is associated with both vaccine escape and resistance to 3TC and other □-nucleoside analogues (37,46,47,51,55,56). Interestingly, this triple mutation has not been reported in the Southern African region to date. It could be a matter of time before it is reported; larger studies and data on the vaccination status of research participants are required.

## Clinical and public health significance of RAMs

The spread of these mutations may lead to a rise in drug resistance in treatment naïve chronic HBV infection, representing a substantial challenge for Africa and highlighting an imperative to ensure routine use of TDF in preference to 3TC-based therapy. Although these data provide a preliminary picture of the prevalence of RAMs in some settings, there are no recommendations to stipulate any specific prevalence threshold above which HBV drug resistance mutations represent a significant barrier to successful treatment at a population level, and/or RAM prevalence thresholds that should trigger a switch to alternative first-line therapy. For HIV, surveillance for transmission of RAMs is based on screening recently infected, treatment naive individuals, and classifies drug resistance using thresholds of <5%, 5-15%, and >15% to stratify the risk to public health (73). Similar thresholds and recommendations for HBV could help to underpin the assimilation of epidemiological data and unify treatment approaches.

## TDF resistance

The identification of mutations associated with reduced TDF susceptibility are of concern, as they suggest the potential for increasing prevalence of polymorphisms that confer partial or complete viral escape from a drug that to date has not been widely associated with resistance. There is now potential for increasing selection of TDF resistance as this drug becomes more widely used. However, as a new first line single tablet option incorporating 3TC, TDF and Dolutegravir (‘LTD’) emerges for HIV treatment, surveillance is needed to determine the clinical outcomes for HBV.

If clinically significant, TDF resistance mutations may still represent a particular problem for many African settings, as resource constraints make it unrealistic to provide baseline screening for RAMs, or to monitor patients on treatment with serial viral load measurements. Despite these potential concerns, it has been shown that TDF is effective even in the presence of RAMs and that there is comparable efficacy among 3TC-experienced and NA-naïve patients (74).

**Limitations of current data**

Screening for HBV infection is not routinely performed in many African settings and therefore the true prevalence and characteristics of HBV infection are not known (4,7–10). We identified very few published studies; only a minority of patients had HBV sequencing undertaken, and there were no data from certain regions of Africa. This highlights the substantial problem of HBV neglect in Africa , and a specific blind-spot relating to sequence data (4). Identifying the true prevalence of resistance mutations, and characterising the populations in which these are selected and enriched, is currently not possible due to sparse data and lack of clear descriptions of the denominator population. Most such studies do not perform a truly systematic assessment, but focus on high risk groups – particularly including those with HIV/HBV coinfection: of the 37 studies included here, only one exclusively reported on participants who were HBV mono-infected (68). Although we have made every effort to assimilate the relevant data to build up a regional picture for Africa, the heterogeneity between studies makes it difficult to draw robust conclusions from pooled data. These findings are a reflection of the little attention paid towards the burden of this disease in Africa and the neglect in robust epidemiological data.

Only nine studies undertook a longitudinal approach to detection of drug resistance (8–10,37,38,42,46,48,50). The results of the other 28 studies that undertook a cross-sectional approach could be skewed by the timing of recruitment of study participants, with a risk of under-representation of drug resistance if screening is undertaken only at baseline, and potentially an over-representation if screening is undertaken in patients with HIV coinfection, who are more at risk of advanced disease and prolonged drug exposure. As most of these studies recruited individuals from hospital settings, this raises the latter possibility.

Mutations across the whole genome might be relevant in determining resistance (75). However, most of the included studies analysed only defined genes from within the HBV genome; only two sequenced the whole genome, and these determined consensus sequence. This potentially results in an under-representation of RAMs and VEMs that may be present as low numbers of quasispecies, but could become significant if selected out by exposure to drug or vaccine.

In studies that reported RAMs among treatment naïve individuals, the literature suggests that identification of RAMs and VEM was performed prior to ART initiation. However, we cannot exclude the possibility that that some of these participants had prior ART exposure. Due to the nature of the cohorts that have been studied, most of the RAMs identified were from HIV/HBV coinfected individuals. It is possible that HIV increases the risk of HBV RAMs both in terms of drug exposure, and also as a function of increased HBV viral loads. A study carried out in Italy revealed that patients with HIV coinfection were more likely to harbour the rtM204V mutation and

to show multiple mutations compared to HBV monoinfected patients (76). It would be worth exploring this hypothesis in Africa, as there are currently no such data.

**Challenges and opportunities for Africa**

A major challenge for Africa is to deploy more widespread screening for HBV, to identify and treat infections when required, but also in order to avoid exposure to inappropriate HBV monotherapy in HIV-coinfected individuals starting ART (16). Despite WHO recommendations to monitor for the development of drug resistance once on therapy (6), implementation remains challenging as viral load monitoring and sequencing are both rarely available (7). Development of a cheap rapid test for the detection of the most frequently observed RAMs and VEMs should be considered as potentially cost-effective alternative for Africa. New metagenomic sequencing platforms, such as Illumina and Nanopore, provide the opportunity for whole and deep genome sequencing, which reveal the full landscape of HBV mutations in individual patients, quantify the prevalence of drug resistance mutations among HBV quasi-species, and determine the relationship between these polymorphisms and treatment outcomes (76). Nanopore technology also has the potential to develop into an efficient point of care test that could detect viral infection and coinfection, as well as determining the presence of VEMs and RAMs (77), but is currently limited by cost and concerns about high error rates.

There have been few studies looking at the correlation between genotype, clinical outcomes of disease, response to antiviral therapy and RAMs/VEMs, but none from Africa. Studies outside Africa have shown that genotype A is more prone to immune/vaccine escape mutants, pre-S mutants associated with immune

suppression, drug associated mutations and HCC in HIV/HBV coinfected participants (39,76,78). Studies investigating the role of genotypes in predicting response to antiviral therapy and their association with various types of mutations are urgently needed in Africa, particularly in light of the high frequency of genotype A infection and high population exposure to antiviral agents that have been rolled out over the past two decades as a component of first-line ART.

Existing infrastructure for diagnosis, clinical monitoring and drug therapy for HIV represents an opportunity for linkage with HBV care; particularly in settings of limited resource, joining up services for screening and management of blood-borne virus infection could be a cost-effective pathway to service improvements.

## Conclusions

This review highlights the very limited data that are available from Africa. Scarce resources resulting in lack of diagnostic screening, inconsistent supply of HBV drugs and vaccines, and poor access to clinical monitoring contribute to drug and vaccine resistance, potentially amplifying the risk of ongoing transmission and adding to the long-term burden of HBV morbidity and mortality in Africa. We call for urgent action to gather and analyse better data, particularly representing the HBV monoinfected population, and for improved access to TDF.

HBV RAMs and VEMs have been identified in several African countries among HIV/HBV coinfected and HBV monoinfected patients, before and during treatment with NAs but the data are currently insufficient to allow us to form a clear picture of the prevalence, distribution or clinical significance of these mutations. Overall, the

data we describe suggest a significantly higher prevalence of drug resistance in some African populations than has been described elsewhere, and that is not confined only to drug-exposed populations, highlighting an urgent need for better population screening, assessment of HBV infection before and during therapy, and increasing roll out of TDF in preference to 3TC. At present, TDF accessibility is largely confined to HIV/HBV coinfected individuals; we now need to advocate to make monotherapy available for HBV infected individuals. However, there are uncertainties as to whether its long-term use might result in nephrotoxicity, and potentially in an increase in selection of TDF RAMs. We should ideally aim for the goals of a combined HBV test that includes diagnosis of infection, genotype and presence of RAMs/VEMs; new sequencing platforms such as Nanopore make this technically possible, although cost remains a significant barrier at present. Sustainable long-term investment is required to expand consistent drug and vaccine supply, to provide screening infection and for drug resistance, and to provide appropriate targeted clinical monitoring for treated patients.

**FUNDING**

JM is funded by a Leverhulme Mandela Rhodes Doctoral Scholarship. PCM is funded by a Wellcome Trust Intermediate Fellowship (grant number 110110). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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**SUPPLEMENTARY DATA: (https://doi.org/10.6084/m9.figshare.5774091)** (80)

**Suppl Figures**

**S1 Fig: Flow diagram illustrating identification and inclusion of studies for a systematic review of drug and vaccine resistance mutations in Africa, based on PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) criteria.**

**S2 Fig: HBV drug Resistance Associated Mutations (RAMs) grouped according to genotype.** Data summarised from fourteen studies published between 2009-2017 (inclusive). 21 studies were not represented here as they did not specifically indicate which genotype individuals with RAMs belonged to.

**S3 Fig: Distribution of HBV genotypes and prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins in geno-A and geno-non-A samples.**

Panel A: Distribution of HBV genotypes derived from 35 studies reporting resistance associated mutations (RAMs) in Africa published between 2009 to 2017 (inclusive);

Panel B: Prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins in geno-A and geno-non-A samples. These data are derived from 14 studies of HBV drug resistance in Africa published between 2007 and 2017 (inclusive). 21 studies were not represented here as they did not specifically indicate which genotype individuals with RAMs belonged to. We had more geno-A samples represented than other samples, we therefore combined samples from other genotypes that had RAMs (B, C, D, E, D/E) to form geno-non-A samples. We then compared prevalence of Pol/RT mutation between geno-A samples to geno-non-A samples.

Prevalence of RT/Pol mutations for a specific genotype(geno-A/geno-non-A) was determined by grouping all studies with geno-A/geno-non-A infection that reported a specific mutation; the denominator was the total number of individuals infected with geno-A/geno-non-A from these studies and the numerator was the total number of individuals infected with geno-A/geno-non-A with that specific mutation.

**Suppl Tables**

**S1 Table: Details of search strategy used to identify studies on HBV resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) conducted in Africa.** A: PubMed database; B: SCOPUS and EMBASE database.

**S2 Table: Full details of 37 studies identified by a systematic literature search of HBV resistance associated mutations (RAMs) and vaccine escape mutations (VEMs) from African cohorts published between 2007 and 2017 (inclusive).**

**S3 Table: HBV Pol/RT mutations among treatment-naïve HBV infected patients in Africa from 12 studies published between 2007 and 2017 (inclusive).**

**S4 Table: HBV Pol/RT mutations among treatment-experienced HBV infected patients in Africa, from 25 studies published between 2009 and 2017 (inclusive).**

**Fig 1: Resistant mutations associated with drug, vaccine and immunoglobulin resistance**

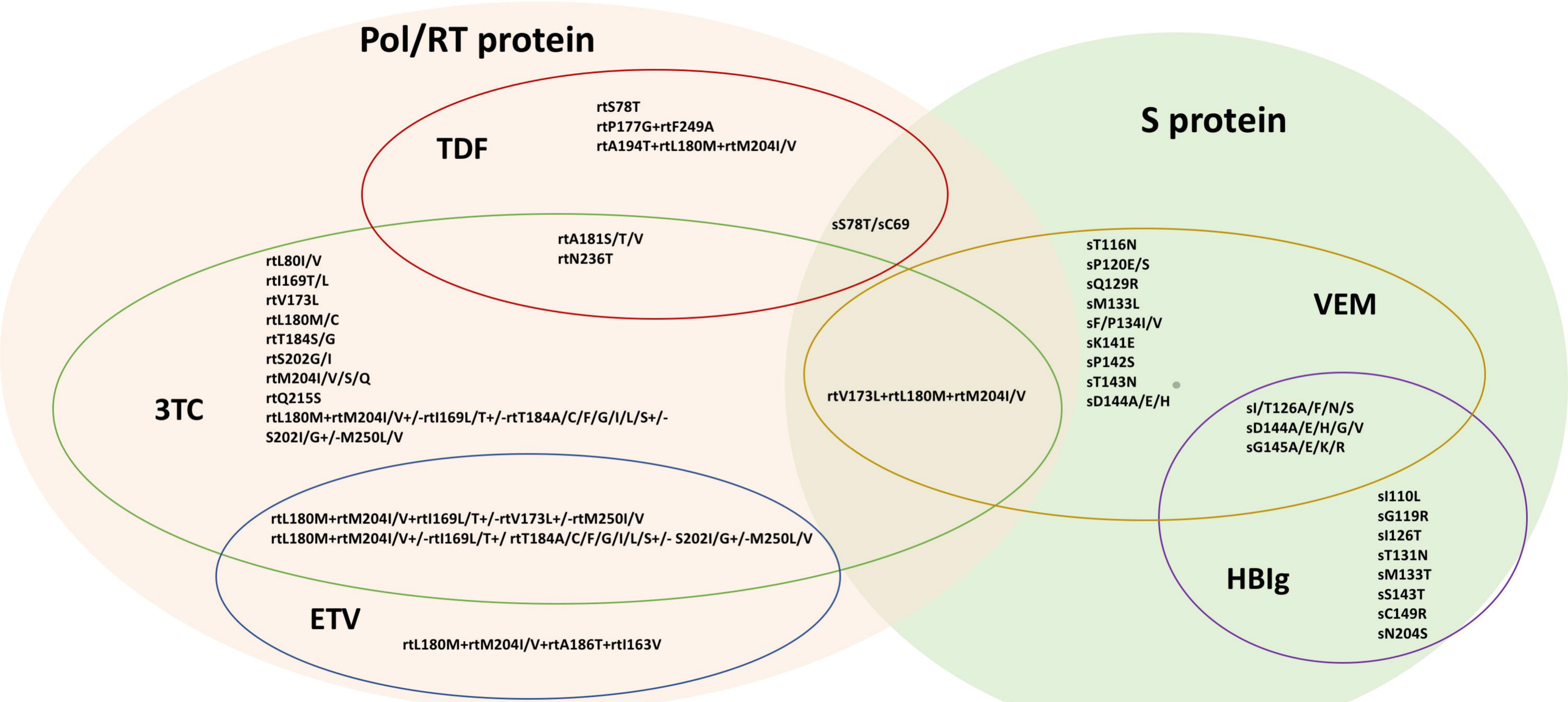



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**Fig 2: HBV drug Resistance Associated Mutations (RAMs) and Vaccine Escape Mutations (VEMs) mutations identified in 33 studies from Africa**

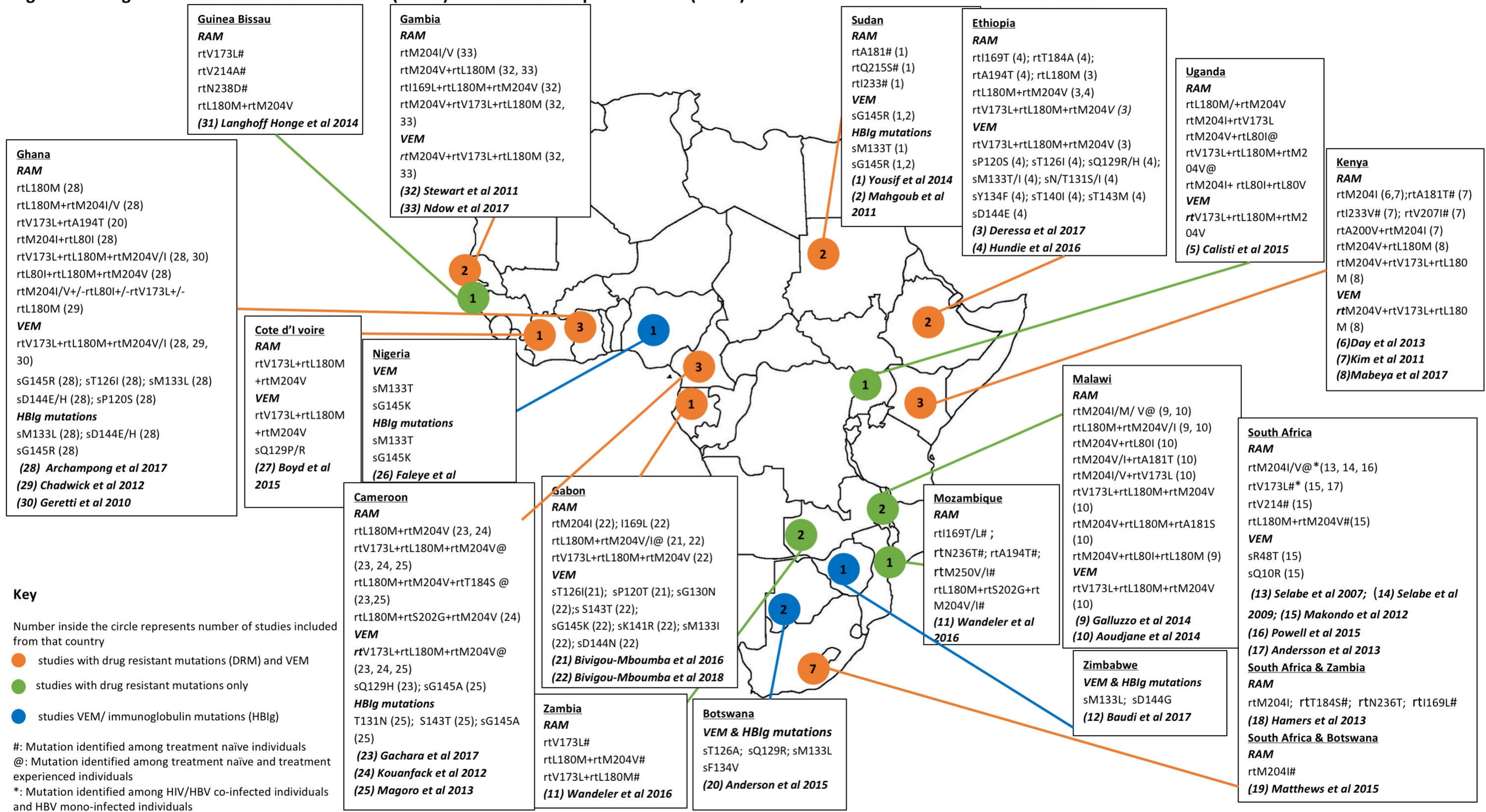
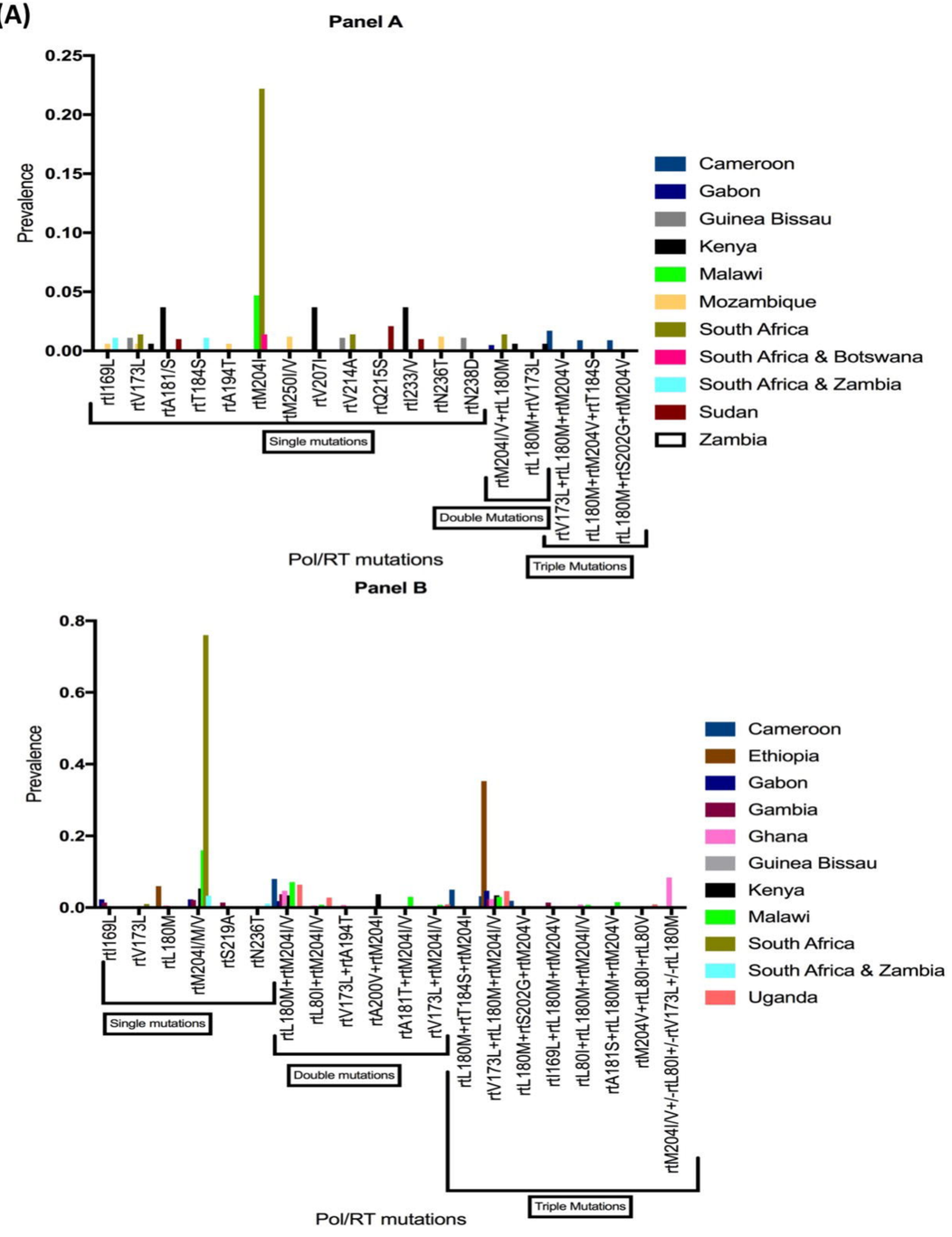
**Fig 3:Prevalence of HBV resistance associated mutations (RAMs) in Pol/RT proteins among HBV infected patients in Africa (A) treatment naïve; (B) treatment experienced**